Dibenzodioxocins in Forage Plant Lignins

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Introduction

Dibenzodioxocins were discovered in plant lignins by a Finnish group. They are exciting firstly because they represent a new structure in lignin that had not been discovered in many decades of research. Furthermore, they explain why analytical methods to quantify non-cyclic α-aryl ether units in lignins claim significant amounts (6-9%) of such structures when diagnostic NMR experiments have shown there to be typically less than 0.3%! As it turns out, the wet chemical analytical methods drew contributions for such cyclic structures. The importance lies in the fact that non-cyclic α-aryl ethers have been ascribed as important branching points in the lignin polymer formed by non-radical reactions; dibenzodioxocins do not fill that role in the same manner.

Utilizing NMR it is possible to identify dibenzodioxocins in a wide variety of lignified materials. They can be difficult to detect in un-acetylated samples due to the smearing of these peaks caused by subtle shifts in both dimensions, but are readily detected in acetylated samples.

The most important aspect impacting our understanding of forage cell walls is the finding that 5–5-coupled diferulates will also form these structures. As noted in an earlier article, diferulates are enormously important in cross-linking cell walls in grasses and reduce the digestibility of those cell walls.

Experimental

A synthetic lignin was prepared incorporating 5–5-coupled diferulate as described in last year's Research Summaries (http://www.dfrc.wisc.edu/contents.html). NMR conditions for the HSQC spectrum: 750 MHz; Bruker pulse program "invietgs" — a 2D ^{1}H-^{13}C correlation via double INEPT transfer using trim pulses, phase sensitive using echo/antiecho TPPI, gradient selection, decoupling during the acquisition; 4K by 256 increments of 16 scans collected; SW's 11 ppm, 150 ppm giving acquisition FID resolutions of 2 and 110.5 Hz/pt. Processing used Gausian multiplication (GB 0.01, LB -0.3 Hz) in F_2 and cosine-squared bell apodization in F_1.

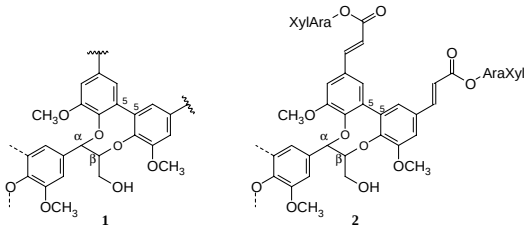

*Figure 1. Dibenzodioxocin structures from 5,5-units in normal lignin **1**, and from 5–5-coupled diferulates in grass lignins **2**.*

Results and Discussion

Utilizing the exceptional power of 750 MHz NMR with pulsed-field gradients, the diferulate dibenzodioxocins of structure 2 are readily identified in HSQC spectra (^{13}C—^{1}H correlation spectra). HMBC and HMQC-TOCSY spectra (not shown) provided further confirmatory evidence by correctly correlating other diagnostic resonances. Unfortunately, due to spectral congestion in this diagnostic area of the NMR spectra, we are currently having trouble authenticating these structures in real forage lignin samples.

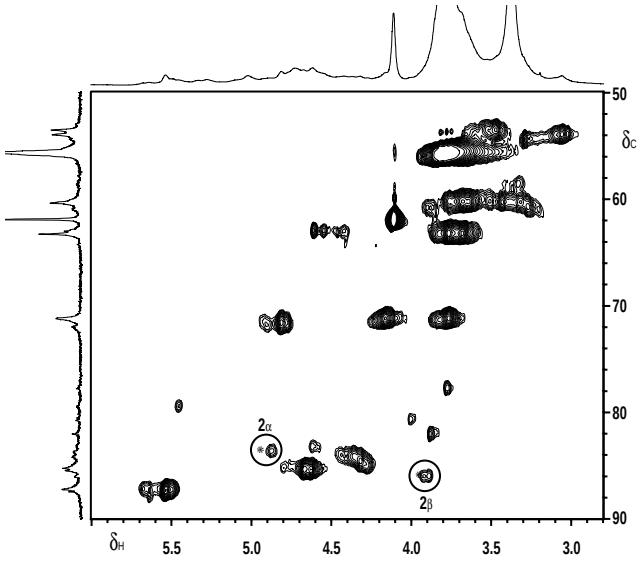

*Figure 2. Partial (sidechain region) 750 MHz gradient-selected HSQC spectrum showing clear evidence for the dibenzodioxocin structures **2**, circled. Correlations from a parent model compound of **2** are shown as *'s.*

The appearance of 5–5-coupled diferulates in these structures helps explain why their releasability is similar to that of monomeric ferulates that are incorporated into lignins. Basically, when one of the ferulate moieties radically couples to C_β of coniferyl or sinapyl alcohol during the early stages of lignification, the phenol on the second moiety becomes tied up by reacting at the α-position of the same lignin monomer. These α,β-di-ethers should be readily released by high-temperature base, or by our new "DFRC" method (see accompanying article).

Identifying these powerful cell wall cross-linking mechanisms helps in our understanding of limitations to plant polysaccharide degradibility by ruminants, and hopefully will eventually lead to rational solutions to the problem.